

Toxic Shock Syndrome Toxin 1 (TSST-1) Production by Staphylococci Isolated from Goats and Presence of Specific Antibodies to TSST-1 in Serum and Milk

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The ability of staphylococcal strains isolated from different anatomical sites in 133 healthy goats to produce toxic shock syndrome toxin 1 (TSST-1) and the presence of antibodies to this toxin in serum and milk were studied. The enzyme-linked immunosorbent assay method was used to detect both the toxin and the presence of antibodies. Of a total of 342 staphylococcal strains studied, 86 (25.2%) were found to produce TSST-1. Specific antibodies to TSST-1 were found in the serum of 57 (42.9%) of the animals studied and the milk of 63 (47.4%) of the animals. These results suggest that goats are frequently in contact with staphylococci that produce TSST-1, a toxin usually associated with *Staphylococcus aureus* strains isolated from cases of toxic shock syndrome in humans.

Toxic shock syndrome (TSS), a systemic illness caused by *Staphylococcus aureus*, was first described by Todd et al. (29) and is presumed to be produced mainly by a staphylococcal protein originally labeled enterotoxin F (2) and pyrogenic exotoxin C (26) and later called toxic shock syndrome toxin 1 (TSST-1) (3). This protein is synthesized by almost all menstrual (2, 4) and many nonmenstrual (13, 26) strains of *S. aureus* isolated from patients with the clinical manifestations of TSS, although it is likely that staphylococcal enterotoxins other than TSST-1 play an important role in the disease (6, 13, 25). The protein is also synthesized by *S. aureus* strains from healthy subjects (15, 20, 24, 27). The fact that no anti-TSST-1 antibodies were found in TSS patients (1), whereas healthy subjects had a high percentage of antibodies (15, 20, 24, 27, 32), seems to indicate that subjects with no antibodies to TSST-1 are more prone to the syndrome than those with antibodies (20).

There are few reports concerning the toxinogenic capacity of staphylococci isolated from animals. TSST-1-producing *S. aureus* has been isolated from poultry (10), goat udder dermatitis and nasal mucosa (19), and mastitic milk of cows (16) and goats (7). In this study, the ability of staphylococci isolated from different body sites in healthy goats to produce TSST-1 was examined, and the presence of antibodies against the toxin in the serum and milk of these animals was determined.

A total of 133 healthy female goats were examined. Sterile swabs were rubbed on the nasal mucosa and the skin of the axilla, udder, and nipple. Milk (25 ml from each compartment) was collected aseptically in sterile containers.

Blood-collecting needles and silicone-coated tubes (Venject; Terumo Co., Leuven, Belgium) were used for blood sampling. Sera were aseptically obtained and transferred to polystyrene sterile tubes, which were then stored at -20°C .

Mucosa and skin samples were streaked onto sheep blood agar (5%) (Oxoid Ltd., Hampshire, United Kingdom). Milk (100 μl) was surface spread on sheep blood agar (5%), and

the remaining milk was frozen at -20°C for subsequent anti-TSST-1 detection. Plates were incubated at 37°C for 24 to 48 h. Colonies whose cell morphology resembled that of gram-positive, catalase-positive cocci were selected, and one or two colonies were subcultured on brain heart infusion agar (Difco Laboratories, Detroit, Mich.) in order to obtain pure cultures.

Identification procedures have been described elsewhere (31). In brief, coagulase-positive strains were identified according to the criteria proposed by Devriese and Hájek (8), and coagulase-negative strains were identified according to the criteria suggested by Devriese et al. (9).

A 500- μl sample of the 18-h broth inoculated with the staphylococcal strains was poured onto the surface of cellophane membranes (Spectrapor membrane tubing; Spectrum Medical Industries Inc., Los Angeles, Calif.) containing brain heart infusion agar, as described by Hallander (14), and harvested after 24 h with 3.0 ml of 0.01 M Na_2HPO_4 .

TSST-1 present in extracts was detected by the enzyme-linked immunosorbent assay (ELISA) method described by Freed et al. (12), with a detection limit of 0.625 ng of TSST-1 per ml. TSST-1 was purified according to the procedure described by Parsonnet et al. (22), and antibodies were raised in New Zealand White rabbits by inoculating them with the purified protein according to the method proposed by Parsonnet et al. (23). To remove staphylococcal protein A, samples or extracts were diluted 1:1 with normal rabbit serum containing inactivated complement (11). The serum-sample mixture was incubated at 37°C for 1 h.

Antibodies against TSST-1 were detected by a competitive method based on the ELISA technique described by Freed et al. (12). Purified TSST-1 was added to serum or milk samples to a final concentration of 10 ng/ml. The mixture was incubated for 1 h at 37°C and stored overnight at 4°C . For the ELISA, (i) serum or milk and (ii) the same serum or milk with TSST-1 added the previous day as described above were incubated (100 μl per well) in the same specific-antibody-coated plates. The presence of antibodies was determined by the difference in optical density between the unsupplemented sample and the corresponding sample with TSST-1 added.

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TABLE 1. Toxigenicity (TSST-1) of staphylococci according to site of isolation

Location	No. (%) of isolates		No. (%) ^a of coagulase-positive strains		No. (%) ^b of coagulase-negative strains	
	Total	Producing TSST-1	Producing TSST-1	Not producing TSST-1	Producing TSST-1	Not producing TSST-1
Nasal mucosa	36	8 (22.2)	4 (57.2)	3	4 (13.8)	25
Axilla	21	0 (0.0)			0 (0.0)	21
Udder skin	88	16 (18.2)	6 (60.0)	4	10 (14.7)	68
Nipple skin	154	43 (28.0)	15/1 ^c (46.9)	17	28 (23.0)	94
Milk	43	19 (44.2)	16 (76.2)	5	3 (13.6)	19
Total	342	86 (25.2)	41 (58.6)	29	45 (16.5)	227

^a Percentage of total number of coagulase-positive strains that produce TSST-1.

^b Percentage of total number of coagulase-negative strains that produce TSST-1.

^c 15 total strains, 1 coagulase-positive strain.

Of a total of 342 staphylococcal strains isolated from different body sites, 86 (25.2%) produced TSST-1; 41 of 70 (58.6%) were coagulase-positive strains, and 45 of 272 (16.5%) were coagulase-negative strains (Table 1). There are few reports on TSST-1 production by *S. aureus* isolated from animals (7, 10, 16, 19), probably because this toxin has usually been associated with human *S. aureus*. In studies with goats, TSST-1-producing *S. aureus* has always been isolated from pathological conditions such as dermatitis (24) or mastitic milk (7), while Jones and Wieneke (16) reported the isolation of toxinogenic strains in pure culture from severe cases of bovine mastitis. In all these studies, the authors found a high percentage of animals harboring TSST-1-producing staphylococci, usually in association with staphylococcal enterotoxin C. Many (79%) of the TSST-1-producing strains isolated by us also produced enterotoxin C (30). We have isolated TSST-1-producing strains from all the sites studied except the axillar fold (Table 1). However, the highest percentage came from the mammary area and from milk, which were the sources of 90.7% of the total isolates.

In addition, in most reports *S. aureus* is the strain usually associated with the production of TSST-1, while in this study 16.5% of the coagulase-negative strains were determined to be TSST-1 producers (Table 1). The ability to synthesize TSST-1 has been described for human coagulase-negative strains identified as *Staphylococcus epidermidis* (2, 5, 17), although some authors do not agree with these results (18, 21). Many of the strains belonging to certain coagulase-negative species isolated in the present study proved to be toxinogenic as determined by the technique that we used (Table 2). Although the technique used for coagulase analysis made use of a conventional coagulase test tube (8), it was not the only method used for identifying coagulase-negative species (31); their characterization was the result of thorough analysis by a variety of methods which followed the criteria proposed by Devriese et al. (9).

Specific antibodies were found in the serum of 57 (42.9%) and in the milk of 63 (47.4%) of the 133 animals studied. Of 57 animals from which TSST-1-producing staphylococci were isolated, antibodies were found in the serum of 25 (43.9%) and in the milk of 32 (56.0%). Of 76 animals from which no TSST-1-producing staphylococci were isolated, antibodies to TSST-1 were found in the serum of 32 (42.1%) and in the milk of 31 (40.8%). Thus, there was no significant difference between the percentage of antibodies detected in carriers and the percentage of antibodies detected in noncarriers of toxin-producing staphylococcal strains. Several reports have shown that a high percentage of healthy humans

have specific anti-TSST-1 antibodies associated with the isolation of toxin-producing *S. aureus* from the nasal mucosa (15, 20, 24). Notermans et al. (20) suggest that individuals lacking specific anti-TSST-1 antibodies may be more susceptible to TSS than those with antibodies. So far, the presence of anti-TSST-1 antibodies in animals has not been studied, paralleling the low number of studies of TSST-1 production by staphylococcal isolates from nonhuman species. However, in the goats sampled in this experiment, a high percentage of TSST-1-producing staphylococci was isolated (Tables 1 and 2) and a high number of animals having specific antibodies detected both in serum and in milk were identified. In a study using commercial cow's milk, Thompson et al. (28) detected a high incidence of antibodies against TSST-1, sometimes at high titers.

The results regarding the isolation of TSST-1-producing staphylococci and the presence of specific antibodies in the milk and serum of the healthy goats studied suggest that these animals may be frequently exposed to TSST-1-producing strains, either habitually or through infection. It was concluded that a study of the role of this toxin in infectious processes in animals and of the role that these animals, as frequent carriers of this type of staphylococci, may have in human health would be of great value.

TABLE 2. TSST-1 production by identified staphylococcal species

Species	No. (%) of strains	
	Total	Producing TSST-1
<i>S. aureus</i>	64	40 (62.5)
<i>S. hyicus</i>	13/6 ^a	1 ^b (7.7)
<i>S. chromogenes</i>	23	3 (13.0)
<i>S. haemolyticus</i>	64	15 (23.4)
<i>S. warneri</i>	45	7 (15.5)
<i>S. epidermidis</i>	32	4 (12.5)
<i>S. caprae</i>	18	3 (16.6)
<i>S. xylosus</i>	23	6 (26.0)
<i>S. sciuri</i>	20	1 (5.0)
<i>S. saprophyticus</i>	13	5 (38.5)
<i>S. cohnii</i>	6	0 (0.0)
<i>S. lentus</i>	3	0 (0.0)
<i>S. equorum</i>	3	0 (0.0)
<i>S. kloosii</i>	3	0 (0.0)
Unidentified ^c	12	1 (8.3)

^a 13 total strains, 6 coagulase-positive strains.

^b Coagulase-positive strain.

^c Coagulase-negative strains.

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REFERENCES

- Bergdoll, M. S. 1985. The toxic shock syndrome toxin-one (TSST-1) and toxic shock syndrome. *Zentralbl. Bakteriol. Suppl.* **14**:91-94.
- Bergdoll, M. S., B. A. Crass, R. F. Reiser, R. N. Robbins, and J. P. Davis. 1981. A new staphylococcal enterotoxin, enterotoxin F, associated with toxic shock syndrome *Staphylococcus aureus* isolates. *Lancet* **i**:1017-1021.
- Bergdoll, M. S., and P. M. Schlievert. 1984. Toxic shock syndrome toxin. *Lancet* **ii**:691.
- Bonventre, P. F., L. Weckbach, J. Staneck, P. M. Schlievert, and M. Thompson. 1983. Production of staphylococcal enterotoxin F and pyrogenic exotoxin C by *Staphylococcus aureus* isolates from toxic shock syndrome-associated sources. *Infect. Immun.* **40**:1023-1029.
- Crass, B. A., and M. S. Bergdoll. 1986. Involvement of coagulase-negative staphylococci in toxic shock syndrome. *J. Clin. Microbiol.* **23**:43-45.
- Crass, B. A., and M. S. Bergdoll. 1986. Involvement of staphylococcal enterotoxins in nonmenstrual toxic shock syndrome. *J. Clin. Microbiol.* **23**:1138-1139.
- De Buyser, M. L., F. Dilasser, R. Hummel, and M. S. Bergdoll. 1987. Enterotoxin and toxic shock syndrome toxin-1 production by staphylococci isolated from goat's milk. *Int. J. Food Microbiol.* **5**:301-309.
- Devriese, L. A., and V. Hájek. 1980. Identification of pathogenic staphylococci isolated from animals and foods derived from animals. *J. Appl. Bacteriol.* **49**:1-11.
- Devriese, L. A., K. H. Schleifer, and G. O. Adegoke. 1985. Identification of coagulase negative staphylococci from farm animals. *J. Appl. Bacteriol.* **55**:45-55.
- Evans, J. B., G. A. Ananaba, C. A. Pate, and M. S. Bergdoll. 1983. Enterotoxin production by atypical *Staphylococcus aureus* from poultry. *J. Appl. Bacteriol.* **54**:257-261.
- Fey, H., H. Pfister, and O. Rüegg. 1984. Comparative evaluation of different enzyme-linked immunosorbent assay systems for the detection of staphylococcal enterotoxins A, B, C, and D. *J. Clin. Microbiol.* **19**:34-38.
- Freed, R. C., M. L. Evenson, R. F. Reiser, and M. S. Bergdoll. 1982. Enzyme-linked immunosorbent assay for detection of staphylococcal enterotoxins in foods. *Appl. Environ. Microbiol.* **44**:1349-1355.
- Garbe, P. L., R. J. Arko, A. L. Reingold, L. M. Graves, P. S. Hayes, A. W. Hightower, F. W. Chandler, and C. V. Broome. 1985. *Staphylococcus aureus* isolates from patients with nonmenstrual toxic shock syndrome. *JAMA* **253**:2538-2542.
- Hallander, H. O. 1965. Production of large quantities of enterotoxin B and other staphylococcal toxins on solid media. *Acta Pathol. Microbiol. Scand.* **63**:299-305.
- Jacobson, J. A., E. M. Kasworm, B. A. Crass, and M. S. Bergdoll. 1986. Nasal carriage of toxigenic *Staphylococcus aureus* and prevalence of serum antibody to toxic shock syndrome toxin 1 in Utah. *J. Infect. Dis.* **153**:356-359.
- Jones, T. O., and A. A. Wieneke. 1986. Staphylococcal toxic shock syndrome. *Vet. Rec.* **119**:435-436.
- Kahler, R. C., J. M. Boyce, M. S. Bergdoll, W. R. Lockwood, and M. R. Taylor. 1986. Toxic shock syndrome associated with TSST-1 producing coagulase-negative staphylococci. *Am. J. Med. Sci.* **292**:310-312.
- Kreiswirth, B. N., P. M. Schlievert, and R. P. Novick. 1987. Evaluation of coagulase-negative staphylococci for ability to produce toxic shock syndrome toxin 1. *J. Clin. Microbiol.* **25**:2028-2029.
- Morgan, K. L., E. Gruffyd-Jones, A. A. Wieneke, J. Azavedo, P. J. Carroll, and L. P. Stevenson. 1986. Staphylococcal toxic shock syndrome. *Vet. Rec.* **119**:559.
- Notermans, S., W. J. Van Leeuwen, J. Dufrenne, and P. D. Tips. 1983. Serum antibodies to enterotoxins produced by *Staphylococcus aureus* with special reference to enterotoxin F and toxic shock syndrome. *J. Clin. Microbiol.* **18**:1055-1060.
- Parsonnet, J., A. E. Harrison, S. E. Spencer, A. Reading, K. C. Parsonnet, and E. H. Kass. 1987. Nonproduction of toxic shock syndrome toxin 1 by coagulase-negative staphylococci. *J. Clin. Microbiol.* **25**:1370-1372.
- Parsonnet, J., R. K. Hickman, D. D. Eardley, and G. B. Pier. 1985. Induction of human interleukin 1 by toxic shock syndrome toxin-1. *J. Infect. Dis.* **51**:514-522.
- Parsonnet, J., J. T. Mills, Z. A. Gillis, and G. B. Pier. 1985. Competitive, enzyme-linked immunosorbent assay for toxic shock syndrome toxin 1. *J. Clin. Microbiol.* **22**:26-31.
- Ritz, H. L., J. J. Kirkland, G. G. Bond, E. K. Warner, and G. P. Petty. 1984. Association of high levels of serum antibody to staphylococcal toxic shock antigen with nasal carriage of toxic shock antigen-producing strains of *Staphylococcus aureus*. *Infect. Immun.* **43**:954-958.
- Schlievert, P. M. 1986. Staphylococcal enterotoxin B and toxic shock syndrome toxin-1 are significantly associated with nonmenstrual TSS. *Lancet* **i**:1149-1150.
- Schlievert, P. M., K. N. Shands, B. B. Dan, G. P. Schmid, and R. D. Nishimuri. 1981. Identification and characterization of an exotoxin from *Staphylococcus aureus* associated with toxic shock syndrome. *J. Infect. Dis.* **143**:509-516.
- Solino Noieto, A. L., E. da Costa Cesar, and M. S. Bergdoll. 1986. Antibodies to staphylococcal enterotoxins and toxic shock syndrome toxin 1 in sera of patients and healthy people in Rio de Janeiro, Brazil. *J. Clin. Microbiol.* **24**:809-811.
- Thompson, N. E., E. Gómez-Lucía, and M. S. Bergdoll. 1986. Incidence of antibodies reactive with toxic shock syndrome toxin 1 in bovine milk. *Appl. Environ. Microbiol.* **51**:865-867.
- Todd, J., M. Fishaut, F. Kapral, and T. Welch. 1978. Toxic shock syndrome associated with phage group-I staphylococci. *Lancet* **ii**:1116-1119.
- Valle, J., E. Gomez-Lucia, S. Piriz, J. Goyache, J. A. Orden, and S. Vadillo. 1990. Enterotoxin production by staphylococci isolated from healthy goats. *Appl. Environ. Microbiol.* **56**:1323-1326.
- Valle, J., S. Píriz, R. De La Fuente, and S. Vadillo. Staphylococci isolated from healthy goats. *J. Vet. Med. Ser. B*, in press.
- Vergeront, J. M., S. J. Stolz, B. A. Crass, D. B. Nelson, J. P. Davis, and M. S. Bergdoll. 1983. Prevalence of serum antibody to staphylococcal enterotoxin F among Wisconsin residents: implications for toxic-shock syndrome. *J. Infect. Dis.* **148**:692-698.